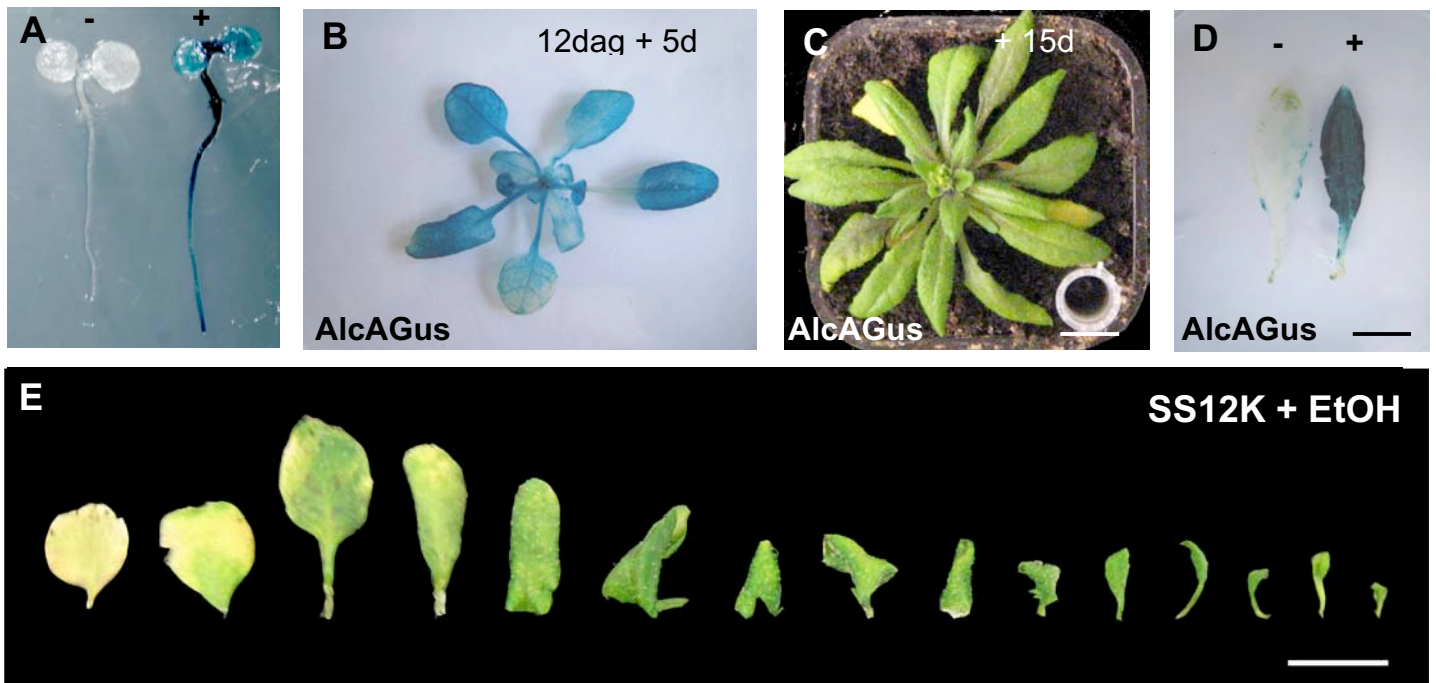


Supplemental Data Braun et al. (2008) Conditional Repression of AUXIN BINDING PROTEIN 1 Reveals That It Coordinates Cell Division and Cell Expansion during Post-Embryonic Shoot Development in Arabidopsis and tobacco.



Supplemental Figure 1. Ethanol induction on AlcAGus control plants and Leaf Growth Decreases after Inactivation of ABP1

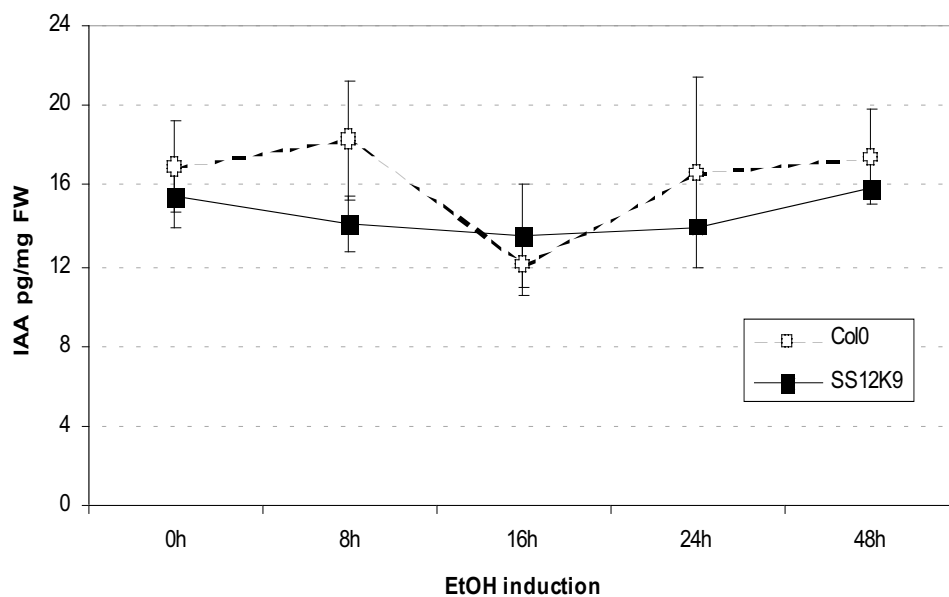
A) AlcAGus 5 day-old seedlings grown in vitro in the presence (+) or absence (-) of 5% EtOH vapour.

B) Gus staining of a 17 day-old AlcAGus plant grown on soil for 12 days without induction and exposed to ethanol vapours for the last 5 days.(similar as figure 2)

C) Rosette phenotype of AlcAGus (27d), 15d after EtOH exposure

D) Gus staining on excised leaves from 27day-old AlcAGus rosettes exposed (+) or not (-) to EtOH for the last 15d.

E) Successive leaves of EtOH-induced SS12K plant (without cotyledons). Exposure to EtOH vapour was performed at the emrgence of leaf 6 as indicated with *. Bar = 1cm.



Supplemental Figure 2. Analysis of IAA accumulation in Col0 and SS12K shoots after various times of ethanol induction as indicated.

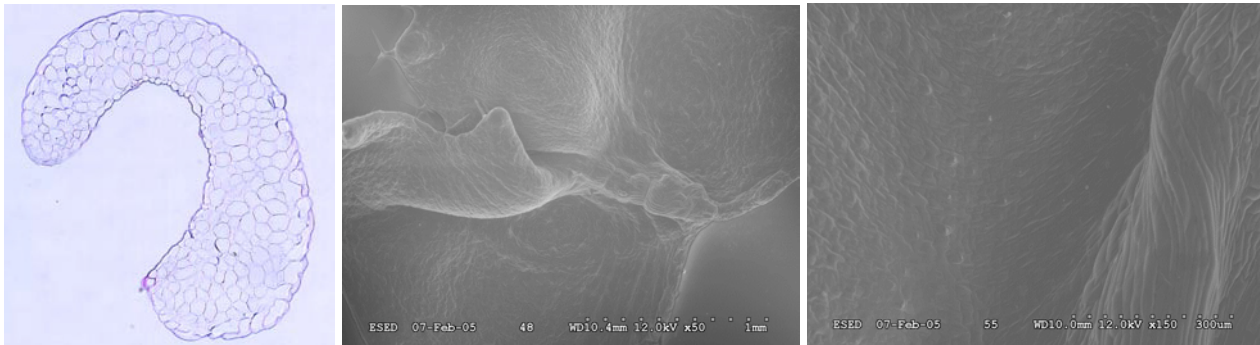
Methods: IAA quantification

Shoots were collected from Col0 and SS12K 4 dpg seedlings induced with ethanol for various times and frozen immediately in liquid nitrogen. The frozen samples were homogenized in 0.5 ml 50 mM sodium-phosphate buffer pH 7.0 containing 0.02% diethyldithiocarbamic acid (Sigma) and 500 pg [¹³C₆]IAA (Cambridge Isotope Laboratories, Andover, MA, USA) internal standard for 2 min at a frequency of 30 Hz, using a Retsch MM 301 vibration mill (Retsch GmbH, Haan, Germany) and a 3 mm tungsten carbide bead. The samples were then incubated for 15 min at +4°C under continuous shaking. The pH was adjusted to 2.7 with 1 M HCl, and the samples were purified by solid phase extraction on a 500 mg Isolute C8 (EC) column (International Sorbent Technology), conditioned with 2 ml methanol and 2 ml 1% acetic acid. After sample application, the column was first washed with 2 ml 10% methanol in 1% acetic acid and then eluted with 2 ml 70% methanol in 1% acetic acid. The dried samples were dissolved in 0.2 ml 2-propanol and 1 ml dichloromethane and 5 µl 2 M trimethylsilyl-diazomethane in hexane (Aldrich) was added to methylate the samples. After methylation, the samples were trimethylsilylated and IAA was quantified by gas chromatography-selected reaction monitoring-mass spectrometry as described in (Edlund et al., 1995). All samples were analysed in triplicates from two biological repeats.



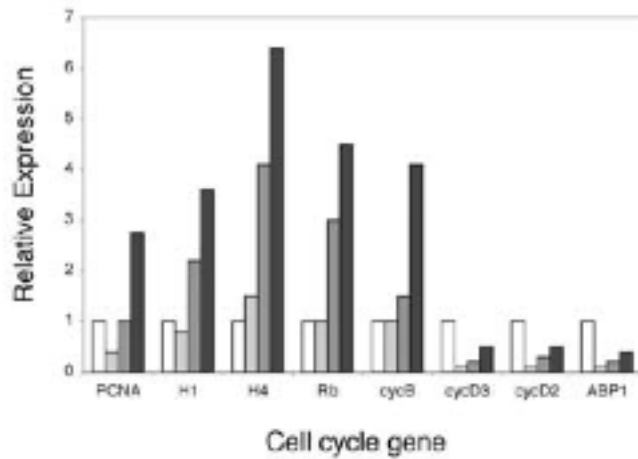
Supplemental Figure 3. Effect of ABP1 inactivation during vegetative growth and flowering

- A) Experimental disposal for ethanol induction of Arabidopsis plants growing on soil
- B) Phenotype of AtSS12K and AtABP1AS lines induced after floral induction
- C-D) detail of induced flowering plants
- E-F) detail of Wt (E) and AtSS12K (F) flower from ethanol induced plants



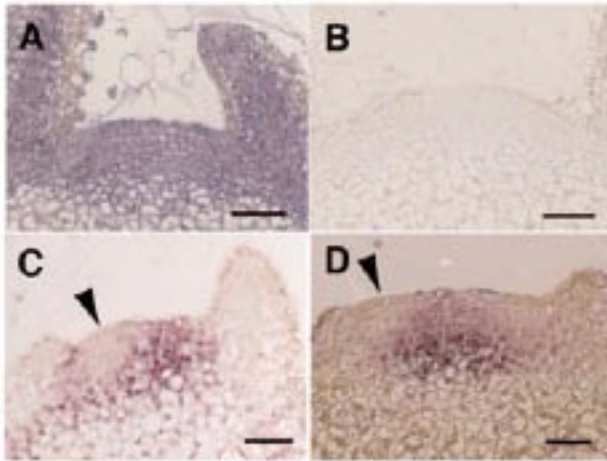
Supplemental Figure 4. Leaf abnormalities resulting from ABP1 inactivation in Arabidopsis

- A) Transverse section at the tip of AtSS12K induced leaf
- B) SEM: occurrence of "double" vascular mid-ribs at the tip of an AtSS12K induced leaf
- C) SEM: twisted mid vein on a AtSS12K induced leaf



Supplemental Figure 5. Cell Cycle Transcript Response to Repression of ABP1 in Tobacco Shoot Apex

Quantitative RT-PCR analysis of NtSS12S7 apices at different times after induction with Ahtet. Primers were used to detect genes as indicated. Signal is shown relative to that for 18s rRNA. Open bars = 0h, light gray bars = 2h, dark gray bars = 8h, black bars = 24h after Ahtet treatment.



Supplemental Figure 6. Local Repression of ABP1 Activity Leads to Altered NTH15 Transcript Pattern

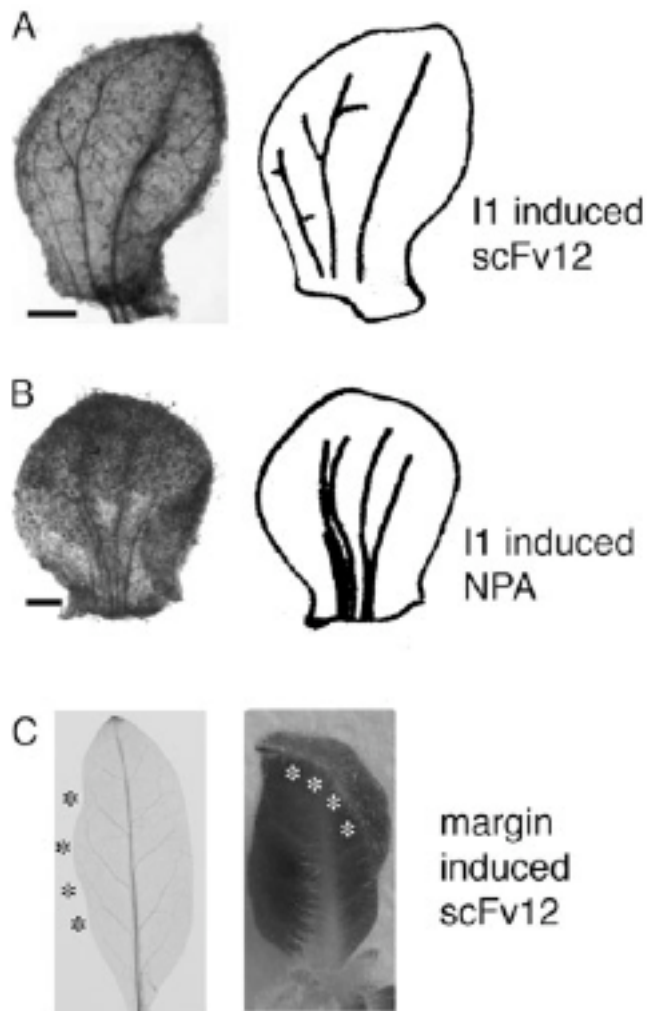
A) Longitudinal section through a WT tobacco apex hybridised with an antisense probe for NtABP1. Signal (purple) is seen throughout the meristem and leaf primordia.

B) As in (A) but hybridised with a sense probe. No signal above background is visible.

C) As in (A) but hybridised with an antisense probe for *NTH15*. Signal (purple) is present throughout the meristem but is absent from the site of incipient leaf formation (arrowhead).

D) As in (C) but with a section from a NtSS12S6 apex in which the meristem has been induced 48 hours previously at the I1 position (arrowhead). Two regions showing lack of transcript signal are apparent within the meristem.

Bars: A and C= 50 μ m; B and D = 25 μ m



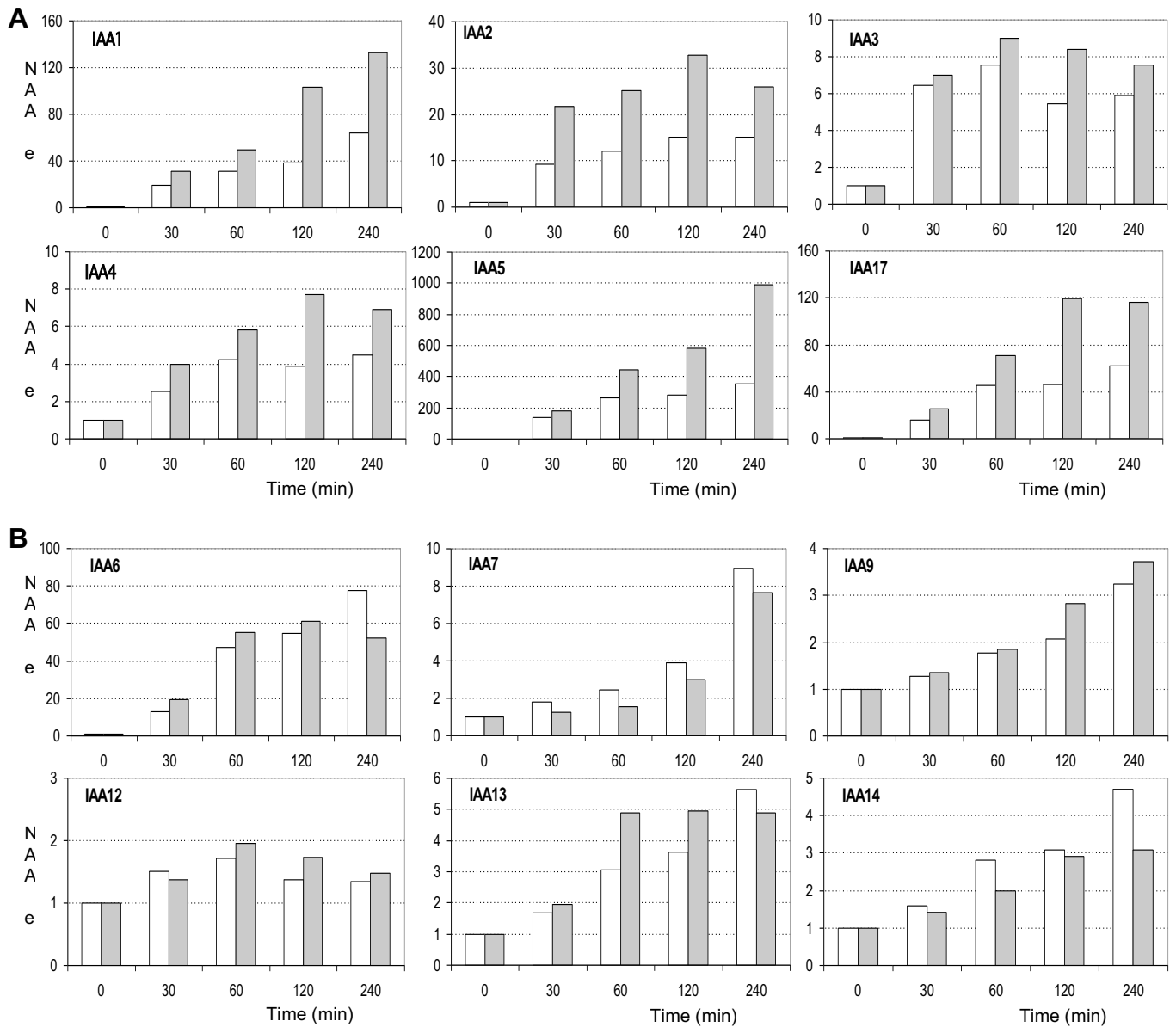
Supplemental Figure 7. Form and Vasculature of Leaves derived from an ABP1 repressed I1 Position on the SAM

A) Primordium derived from the induced I1 position of NtSS12S6 SAMs and cleared to reveal the vascular pattern. The right hand panel shows a line drawing to highlight the observed vascular pattern.

B) Primordium derived from NtSS12S6 SAMs treated at the I1 position with NPA and cleared to reveal the vascular pattern. The right hand panel shows a line drawing to highlight the observed vascular pattern.

C) Leaves from NtSS12S6 plants in which the margins of P2-P3 stage primordia were induced with AhTet. Site of induction is indicated by asterisks (*).

Bars = 2mm.



Supplemental Figure 8. Altered response of *Aux/IAA* genes to auxin after inactivation of ABP1

Kinetic effects of 5µM NAA treatment on transcript accumulation of *Aux/IAA* genes as indicated in 4 day-old seedlings induced overnight with ethanol prior to auxin application. Open bars represent AlcAGus control seedlings and grey bars are SS12K. Data were normalized with *ACTIN2-8* then to the expression level at time zero of auxin application.

Supplemental Table 1. List of primers used for QRT-PCR analysis in Arabidopsis.

	Forward primers	Reverse primers
<i>ACTIN2</i> - <i>ACTIN8</i>	GGT AAC ATT GTG CTC AGT GGT GG	AAC GAC CTT AAT CTT CAT GCT GC
<i>IAA1</i>	ACC GAC CAA CAT CCA ATC TC	TGG ACG GAG CTC CAT ATC TC
<i>IAA2</i>	ATC ACC AAC CAA CAT CCA GTC	TGG ACG GAG CTC CAT ATC TC
<i>IAA3</i>	CAA CCC AAG CAC AGA CAG AG	TGA TTG GAT GCT CAT TGG TG
<i>IAA4</i>	CAA CAA TCT GAG CCT TTG GAG	ATT GGG ATT ACC AGG GAC AG
<i>IAA5</i>	TCC AAG GAA CAT TTC CCA AG	CCG GAG AAA GAA CAG TCT CG
<i>IAA6</i>	AAC TGT TGC TCG AAC CAA GG	ACT GCC GGT TGT GAA GAG TC
<i>IAA7</i>	CTT CTC CTT GGG AAC AGC AG	TCG GCC AAC TTA TGA ACC TC
<i>IAA9</i>	GCT GCA GCT AAC CCA ATA GC	GAG CTG CTG GGA AGG ATA TG
<i>IAA10</i>	CCG GAG AAG AAT CAG CAG AG	GAG CTG CTG GGA AGG ATA TG
<i>IAA12</i>	GCT CTT GCT GCC TTC ATA GC	GTT GGG TCT AAA CGC TCT GC
<i>IAA14</i>	CCA AGG CAT CAG AGA GAT CC	GAA GCA GAG GAG GCA ATG AG
<i>IAA17</i>	TTG ATT TTT GGC AGG AAA CC	AGG GTT CTC AGA GAC GGT TG
<i>IAA19</i>	GAC TCG GGC TTG AGA TAA CG	CGT GGT CGA AGC TTC CTT AC
<i>IAA28</i>	TCA AAG CCA AAC CCC ATT AG	TAA AGT TCT GGT CGG GGA TG
<i>ABPI</i>	GCTCCAGGTTTCAGAGACACC	TAA TAG GCG GCC GAG ATA TG
<i>scFv12</i>	GAA ACA GAG GCC TGG ACA AG	CTG CAT GTA GGC TGT GTT GG

Supplemental Table 2. List of primers used for RT-PCR analysis in Tobacco.

	Forward primers	Reverse primers
<i>Nt.PCNA</i>	CTCAAGGCTGACGATGGCAGTG	GGTACTCTGCTTCTGGAATCCC
<i>Nt.H1</i>	AGCCCGGAGCTAAGCCGAAAGC	GCAACAAGCTTCGCCTTCACC
<i>Nt.H4</i>	GAGGAGACCCGTGGTGTGTTG	GCCAAATCCATAGAGAGTCCTTCC
<i>Nt.RBR</i>	GCTGGGTCCGGAAGCTTGTCT	CACCTGTAAGCACAGCGATGACAA
<i>Nt.CYCB</i>	CTCTCACTGCTAGGAGCAAGGC	CCTCAACATATTCCACCACAGCC
<i>Nt.CYCD2</i>	GTCGAGAGATTGAGAAGTGGA	CTCAGCTCTCCAAATCCATAG
<i>Nt.CYCD3</i>	GGCTGATTGTAGATTCGTACG	CTCCTTGTTAATTTGAGAACCCC
<i>Nt.18S</i>	CCGGGCTCTATGAGTCTGGTAATTGG	GGAGCTGGAATTACCGCGGCTGC
<i>ssFv12</i>	CAAATCCTCCAACACAGCCTACATGCAG	GGTCCCTTGGCCCCAGTATGGAAT